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A cryptobiosis-specific 19S protein complex of *Artemia salina* gastrulae

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The postribosomal supernatant of *Artemia salina* cryptobiotic embryos contains a large quantity of a 19S protein complex. An amount of 3.6mg/g of cysts is measured by immunoprecipitation with anti-(19S protein complex) antibody. The quantity of this complex decreases during further development to nauplius larvae to only 15% of the quantity present in cryptobiotic embryos. The complex was no longer detectable after 7 days of growth. The 27000-M_r protein subunit of the 19S complex is not synthesized by mRNA isolated from cryptobiotic embryos. The cryptobiosis-specific complex has M_r 573000 and 610000 as calculated from light-scattering and sedimentation–diffusion measurements respectively. The 19S homocomplex contains 20–23 27000-M_r proteins and has no function in the translation of homologous mRNA. From hydrodynamic data a hydration of 1.25g of water/g of protein is calculated. The abundant presence of the 19S protein complex in cryptobiotic embryos and the absence of synthesis during development to nauplius larvae indicate a functional role during the cryptobiotic process in early embryogenesis. A role in maintaining the water content of the cytoplasm above a critical threshold during desiccation is suggested.

The development of *Artemia salina* cryptobiotic gastrulae to nauplii is accompanied by changes in the pattern of cytoplasmic proteins. As demonstrated by Grosfeld & Littauer (1976), the cytoplasm of dormant embryos contains two predominant proteins of M_r 24000 and 25000, which are absent from nauplius larvae. We have shown previously that a dominant cytoplasmic protein of M_r 27000 constitutes a 19S complex that decreases drastically during the development of dormant gastrulae to free-swimming nauplius larvae (De Herdt *et al.*, 1979*a*). It is likely that the 25000-M_r and 27000-M_r proteins detected in both laboratories are identical.

The RNA-lacking 19S complex is a spherical particle with a buoyant density of 1.31 g/cm^3 in CsCl and is a specific aggregated form of a 27000-M_r protein. This protein is slightly acidic and contains six cysteine residues/molecule through which extensive disulphide bridges are formed (De Herdt *et al.*, 1979*a*). The 27000-M_r protein is antigenically related to *Artemia* eEF-Ts factor but has no eEF-Ts-factor activity *in vitro*. Co-electrophoresis and isoelectric focusing demonstrated that the 27000-M_r protein and eEF-Ts factor are different proteins (De Herdt *et al.*, 1979*a*, 1980; Slobin, 1980).

The 19S complex has RNA-binding properties and is retained on rRNA–Sepharose (De Herdt *et al.*, 1980). It has been shown that RNA-binding proteins include initiation and elongation factors of translation and that they can be incorporated in ribonucleoproteins (Elizarov *et al.*, 1978; Ovchinnikov *et al.*, 1978; Vlasik *et al.*, 1978). The 27000-M_r protein is one of the major RNA-binding proteins of *Artemia salina*, but is not associated with messenger ribonucleoprotein, although a protein with M_r 27000 is associated with small poly(A)-containing messenger ribonucleoprotein (Slegers *et al.*, 1979; De Herdt *et al.*, 1980).

The structural relationship of the 19S complex with *Artemia* eEF-Ts factor and the RNA-binding properties prompted us to characterize this complex in more detail. In the present paper we provide evidence that the 19S complex is specific for the cryptobiotic phase in *Artemia* embryos.

Abbreviations used: eEF factor, eukaryotic elongation factor; eEF-Ts factor, eukaryotic elongation factor analogous to bacterial EF-Ts.

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Materials and methods

Materials

Artemia salina (San Francisco Bay brand) was

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purchased from Metaframe, Newark, CA, U.S.A. Sepharose 4B was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Wimex H & W sea salt was from H. Wiegandt, Krefeld, W. Germany. Globin mRNA was obtained from Searle, High Wycombe, Bucks., U.K. [^{35}S]Methionine was from The Radiochemical Centre, Amersham, Bucks., U.K. Tobacco-mosaic virus was a gift from Dr. T. Ohno, Department of Biochemistry and Biophysics, University of Tokyo, Japan. Anti-(19S protein complex) antibody was prepared in the laboratory of Dr. M. Joniau, Interdisciplinary Research Centre, Kortrijk, Belgium.

Buffers

Buffer A was 35mM-Tris/HCl (pH7.7)/70mM-KCl/5mM-$MgCl_2$, buffer C was 10mM-sodium phosphate (pH6.8)/50mM-NaCl/5mM-$MgCl_2$, buffer P was 50mM-Tris/HCl (pH7.4)/190mM-NaCl/6mM-EDTA/2.5% Triton X-100 and buffer Q was 10mM-sodium phosphate (pH6.8)/100mM-NaCl.

Fractionation of homogenates

Cryptobiotic embryos of *Artemia salina* were separated from sand and metal impurities by flotation in saturated NaCl, washed with distilled water and ground in a mortar with a motor-driven pestle (Retsch, Düsseldorf, West Germany) in the presence of buffer A or C containing 150mM-sucrose. The subsequent fractionation and purification of the 19S protein complex from the postribosomal supernatant were essentially as described previously (De Herdt *et al.*, 1979*a*).

Batches (10–15g) of embryos were incubated at 22°C in artificial sea-water (39g of sea salt/l). Vigorous aeration resulted in hatching after 48h. Nauplii were further cultured in aquaria (20cm × 70cm × 40cm) and fed with an algal homogenate of *Spirulina maxima* (Heip *et al.*, 1978). At different developmental times, embryos of nauplii were homogenized (De Herdt *et al.*, 1979*a*) and fractionated at 4°C by differential centrifugation in the Beckman JA 20 rotor. Cell debris was removed by centrifugation at 100*g* for 1.5min, nuclei at 400*g* for 3min, membranes at 5000*g* for 10min and mitochondria and small membrane fragments at 11300*g* for 30min. Ribosomes were sedimented in the Beckman R 60 rotor at 254000*g* for 50min at 4°C. Each sediment obtained during the fractionation was washed three times with homogenization buffer, and the proteins were extracted with buffer A containing 0.5% Triton X-100 and 0.5% sodium deoxycholate (Dewald *et al.*, 1974).

Protein synthesis

The preparation of poly(A)-containing mRNA and poly(A)-lacking mRNA from *Artemia salina* has been described (De Herdt *et al.*, 1979*b*). Templates were translated in a rabbit reticulocyte lysate prepared as described by Pelham & Jackson (1976).

Anti-(19S protein complex) antibody immunoprecipitation

The preparation of anti-(19S protein complex) antibody is described elsewhere (De Herdt *et al.*, 1979*a*). The immunoglobulin G fraction was further purified by affinity chromatography on 19S protein complex coupled to Sepharose 4B (Kuwano & Takahashi, 1978). The amount of 19S protein complex was measured by immunoprecipitation. The reaction mixture (final volume 500μl) contained 0.1 A_{280} unit of purified anti-(19S protein complex) antibody and 150mM-NaCl and was incubated for 1h at 37°C. After 16h at 4°C the immunoprecipitate formed was collected by centrifugation, washed twice with 150mM-NaCl and dissolved in 0.1M-NaOH. The absorbance at 280nm was measured in a Zeiss PMQ 3 spectrophotometer.

Immunoprecipitation of proteins synthesized in a rabbit reticulocyte lysate was performed in the presence of sodium dodecyl sulphate (Goldman & Blobel, 1978). A 25μl volume of incubated lysate was diluted with 25μl of Tris/HCl buffer, pH7.5, and made 2% (w/v) with respect to sodium dodecyl sulphate. After incubation at 100°C for 2min, the sample was cooled to room temperature. After addition of 300μl of buffer P, 65μg of 19S protein complex and 0.1 A_{280} unit of anti-(19S protein complex) antibody, the mixture was incubated for 1h at 37°C. After 16h at 4°C the precipitate was collected, washed twice with 150mM-NaCl and dissolved in 1ml of 0.1M-NaOH. A solution (500μl) containing 1M-NaOH, 0.5M-H_2O_2 and 1mg of methionine/ml was added. After 15min at 37°C, proteins were precipitated with 10% (w/v) trichloroacetic acid and collected on Whatman GF/C filters. The radioactivity was counted in a Packard Tri-Carb 2450 scintillation spectrometer.

Determination of $A_{280}^{1\%}$

$A_{280}^{1\%}$ was determined by the modified Lowry method as developed by Hartree (1972), with bovine serum albumin as a standard protein with $A_{280}^{1\%}$ 6.6.

The relation between protein concentration and absorbance at 280nm was also determined from the nitrogen content of protein samples measured by the Kjeldahl method (Ballentine, 1957). Ammonia set free by destruction in 18M-H_2SO_4 was distilled into 25ml of 2% H_3BO_3 with a Kjeltic System I 1002 distilling unit (Tecator, Helsinborg, Sweden). The amount of ammonia was determined from potentiometric titrations.

Determination of hydrodynamic parameters

Light-scattering experiments were conducted in a

model 5200 light-scattering duophotometer (Wood, Newtown, PA, U.S.A.) with polarized and unpolarized light at wavelengths of 436 and 546nm. The calibration of the apparatus has been described (De Voeght *et al.*, 1979). Owing to the spherical nature of the particle, the intensity of the scattered light is independent of the angle. The intensity of scattered light at $\theta = 90°$ is compared with the intensity of light scattered by benzene and a standard glass diffuser.

Samples of the 19S complex in buffer Q with concentrations suitable for light-scattering measurements were prepared by Amicon ultrafiltration (Oosterhout, The Netherlands) with an XM 50 filter and a subsequent removal of aggregates by chromatography on Sepharose 4B.

Molecular weights were calculated from the relation:

$$\left(\frac{\mathscr{K}c}{R_{90°}}\right)_{c=0} = \frac{1}{M}$$

with $\mathscr{K}$ an optical constant, $R_{90°}$ the Rayleigh ratio at $\theta = 90°$ and c the concentration of the 19S complex.

Single clipped photon-count autocorrelation spectra were obtained with a set-up of Malvern Instruments (Malvern, Worcs., U.K.) and a coherent-radiation argon ion laser with a wavelength of 488nm. The normalized autocorrelation function was measured at 25°C from the light scattered at $\theta = 45°$, 90° and 135° and is related to the diffusion coefficient D (Foord *et al.*, 1970; Pusey *et al.*, 1974; Koppel, 1974:

$$g_k^{(2)}(iT) - 1 = A \cdot \exp(-2DK^2iT)$$

with $i = 1, 2 \ldots 24$, A an experimental constant, T the sample time and K the scattering vector. The calibration of the set-up has been described (Nieuwenhuysen & Clauwaert, 1977).

The experimental correlation functions were analysed by a linear and quadratic fitting procedure of the function: $\ln[g_k^{(2)}(iT) - 1]$ (Nieuwenhuysen & Clauwaert, 1977).

Samples were prepared as before.

The density increment was obtained from density measurements with a precision density-meter DMA 02C (Anton Paar, Graz, Austria) (Kratky *et al.*, 1973). The temperature was kept constant within 0.005°C with a Tronac model 40 precision temperature controller (Tronac, Orem, UT, U.S.A.). Purified 19S samples were concentrated by Amicon ultrafiltration with an XM 50 filter and dialysed against buffer C.

The refractive-index increment was measured with a Rayleigh–Haber–Löwe interferometer.

Results

Localization and quantity of the 19S complex during development

Cryptobiotic embryos and nauplius larvae were fractionated into nuclear, membrane, mitochondrial, ribosomal and post-ribosomal fractions by differential centrifugation. Proteins were extracted from each fraction with 0.5% (v/v) Triton X-100 and 0.5% (w/v) sodium deoxycholate as described by Dewald *et al.* (1974). This method resulted in a recovery of 70–100% of the membrane proteins. Extracted proteins were tested for the presence of the 19S complex by immunoprecipitation with the anti-(19S protein complex) antibody. Most of the 19S complex was located in the postribosomal supernatant. The amount in other fractions was less than 5% (Fig. 1*a*). No change in localization of the 19S complex was observed after development of cryptobiotic embryos to free-swimming nauplii (Fig. 1*b*).

The amount of 19S complex was measured from the absorbance at 280nm of the alkali-dissolved immunoprecipitate (Fig. 2*a*). Changes in the absolute quantity of the 19S complex during development were measured on embryos homogenized and fractionated at different developmental times. Cryptobiotic embryos (10g) were incubated at 22°C in artificial sea-water for an appropriate time as indicated in Fig. 2(*b*). The amount of 19S complex was measured from the precipitate with anti-(19S protein complex) antibody and is expressed as mg of 19S complex/g of embryos. The quantity of the 19S complex decreased almost linearly during development. In cryptobiotic embryos 3.6mg of 19S complex/g of cysts was measured, which represents almost 50% by weight of the quantity of 80S ribosomes. A molecular ratio of 3:1 for 19S complex/80S ribosomes can be calculated in the dormant stage. Nauplii hatched after 48h contained only 15% of the quantity detected in cryptobiotic gastrulae. After 7 days of growth no 19S complex was detectable by immunoprecipitation.

Synthesis of the 27000-M_r protein of the 19S complex

In the cytoplasm of cryptobiotic embryos, template activity is associated with a poly(A)-lacking 22S messenger ribonucleoprotein, containing 9S mRNA. Both messenger ribonucleoprotein and mRNA code predominantly for a 26000-M_r protein (De Herdt *et al.*, 1979*b*). The poly(A)-containing mRNA of cryptobiotic embryos becomes active as template only after removal of the translational inhibitor oligonucleotide by affinity chromatography on poly(U)–Sepharose (Slegers *et al.*, 1977). The main protein product of poly-(A)-containing mRNA in a rabbit reticulocyte

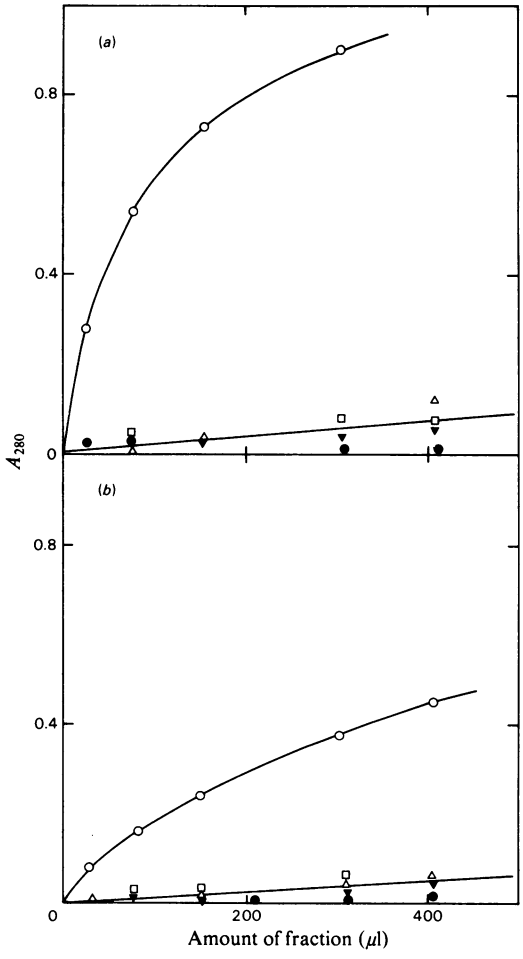

Fig. 1. *Localization of the 19S protein complex*
Cryptobiotic embryos (*a*) and nauplius larvae (*b*) were fractionated into nuclear (●), membrane (□), mitochondrial (▼), ribosomal (△) and post-ribosomal (○) fractions. Proteins of each fraction were extracted with 30ml of buffer A containing 0.5% (v/v) Triton X-100 and 0.5% (w/v) sodium deoxycholate. Increasing amounts were incubated with 0.1 A_{280} unit of anti-(19S protein complex) antibody. The immunoprecipitate was dissolved in 1ml of 0.1M-NaOH and the absorbance measured at 280nm.

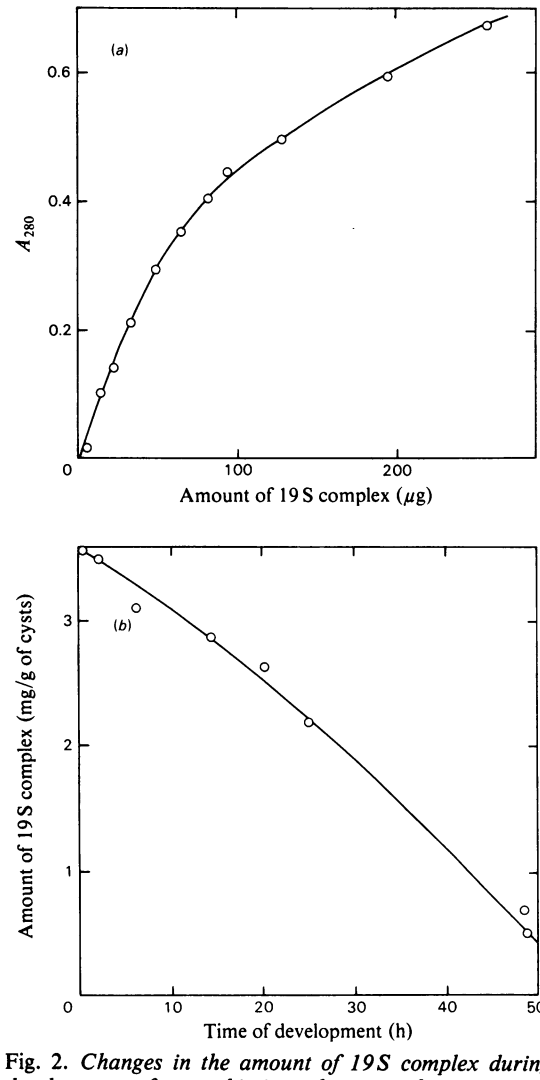

Fig. 2. *Changes in the amount of 19S complex during development of cryptobiotic embryos to free-swimming nauplii*
(*a*) Calibration curve of the 19S protein complex. Increasing amounts of purified 19S complex were precipitated with 0.1 A_{280} unit of anti-(19S protein complex) antibody. The immunoprecipitate was dissolved in 1ml of 0.1M-NaOH and the absorbance measured at 280nm. (*b*) Cryptobiotic embryos (10g) were incubated for the indicated time at 22°C. The amount of 19S complex present in the post-ribosomal supernatant was determined by immunoprecipitation.

protein-synthesizing system also displays a molecular weight of 26000. These proteins synthesized *in vitro* have approximately the same molecular weight as the 27000-M_r protein, which constitutes the 19S complex. However, no synthesized proteins could be precipitated by anti-(19S protein complex) antibody (Table 1). These results demonstrate that the 19S complex is formed before cryptobiosis.

Spectral properties

The spectrum of the 19S protein complex has a minimum at 251nm, a maximum at 280nm and a shoulder at 290nm (Fig. 3). An extensively purified preparation is characterized by an A_{280}/A_{260} ratio of 1.52 and an $A_{max.}/A_{min.}$ ratio of 1.80.

Table 1. *Immunoprecipitation of proteins synthesized in vitro with anti-(19S protein complex) antibody*
Experimental details are indicated in the text.

Template	Fraction of ^{35}S radioactivity		
	Supernatant	Wash	Immunoprecipitate
Tobacco-mosaic-virus RNA	0.95	0.02	0.03
Globin mRNA	0.97	0.01	0.02
Artemia poly(A)-lacking mRNA	0.95	0.02	0.03
Artemia poly(A)-containing mRNA	0.94	0.02	0.04

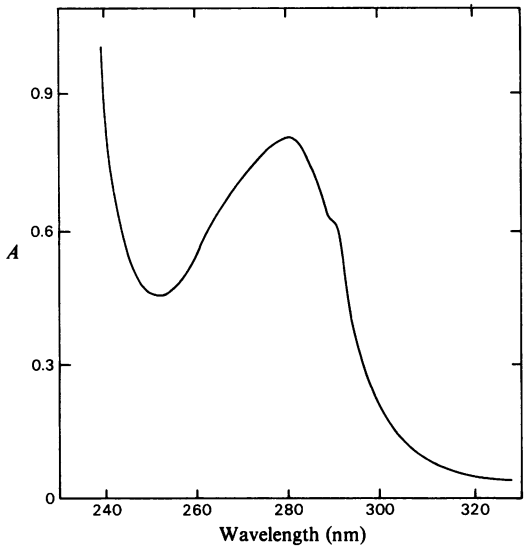

Fig. 3. *U.v.-absorption spectrum of the 19S complex*
The 19S complex was prepared as described in the Materials and methods section and further purified by chromatography on Sepharose 4B. The complex was extensively dialysed against buffer C. The absorbance was measured from 240 to 320nm.

Table 2. *Determination of the absorption coefficient,* $A_{280}^{1\%}$
Experimental details are indicated in the text.

Method		$A_{280}^{1\%}$
Hartree (1972), with bovine serum albumin as standard protein	Expt. 1	15.0 ± 0.6
	Expt. 2	15.4 ± 0.6
	Expt. 3	15.2 ± 0.6
Nitrogen determination by the procedure of Ballentine (1957)	Expt. 1	15.2 ± 0.2
	Expt. 2	15.2 ± 0.2

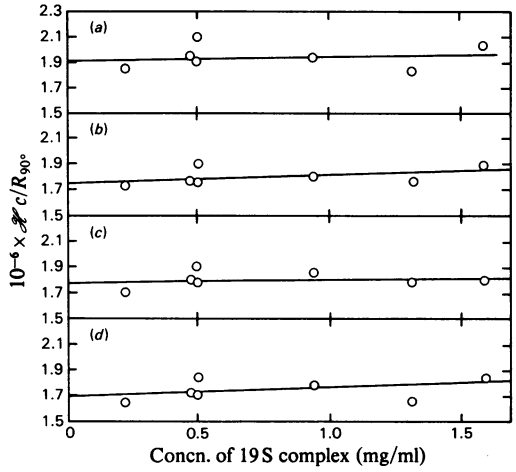

Fig. 4. *Molecular-weight determination of the 19S protein complex by light-scattering, with benzene as standard*
$\mathscr{K}c/R_{90°}$ is plotted as a function of the concentration of 19S complex for different experimental set-ups. (*a*) Polarized light, 436nm; (*b*) unpolarized light, 436nm; (*c*) polarized light, 546nm; (*d*) unpolarized light, 546nm. Purified 19S complex in buffer Q was concentrated by ultrafiltration, and aggregates were removed by chromatography on Sepharose 4B. Peak fractions were freed of dust by centrifugation at 15000 **g** for 2h.

The absorption coefficient, $A_{280}^{1\%}$, was measured by two independent methods: the method of Hartree (1972), with bovine serum albumin as a standard, and a nitrogen determination by the procedure of Ballentine (1957). From the amino acid composition of the 19S complex (De Herdt *et al.*, 1979*a*) a nitrogen content of 16.14% (w/w) was calculated. The measured absorption coefficients are presented in Table 2. The same value of $A_{280}^{1\%} = 15.2 \pm 0.2$ is measured with both methods, although the method of Hartree (1972) depends on the standard protein used.

Molecular-weight determination and hydrodynamic properties

The molecular weight of the 19S complex was obtained from light-scattering experiments at wavelengths of 436 and 546nm and at a scattering angle $\theta = 90°$. A linear plot between $\mathscr{K}c/R_{90°}$ and c was observed with polarized and unpolarized light (Fig. 4). The measured values of $\partial n/\partial c$, 0.191cm^3/**g** at 436nm and 0.185cm^3/**g** at

546 nm, are used to calculate the optical constant $\mathscr{K}$. The molecular weights determined with different experimental set-ups (Table 3) were compared with the value calculated from the Svedberg relation (Eisenberg, 1976):

$$M = \frac{s^0_{20,w} \cdot \boldsymbol{R} \cdot T}{D^0_{20,w} \cdot \left(\frac{\partial \rho}{\partial c}\right)^0_\mu}$$

with $\boldsymbol{R}$ is the gas constant, $T = 293\,K$, $(\partial\rho/\partial c)^0_\mu$ is the density increment, and $s^0_{20,w}$ and $D^0_{20,w}$ are the sedimentation coefficient and diffusion coefficient corrected to standard conditions. A sedimentation coefficient of 19.1 S has been measured previously by analytical ultracentrifugation (De Herdt *et al.*, 1979*a*). Diffusion coefficients were determined by single clipped photon-count autocorrelation spectroscopy of scattered laser light (Foord *et al.*, 1970; Pusey *et al.*, 1974; Koppel, 1974; Nieuwenhuysen & Clauwaert, 1977).

The diffusion coefficient is concentration-dependent, and extrapolation to zero concentration yielded $D^0_{20,w} = (2.72 \pm 0.05) \times 10^{-7}\,cm^2/s$ (Fig. 5). A density increment $(\partial\rho/\partial c)^0_\mu = 0.277 \pm 0.004$ was obtained from the plot of density versus concentration of 19 S complex (Fig. 6). With the relation:

$$\left(\frac{\partial \rho}{\partial c}\right)^0_\mu = 1 - v^0\rho^0$$

a partial specific volume of $0.72\,cm^3/g$ was calculated for the 19 S protein complex.

Substitution of the measured values of $s^0_{20,w}$, $D^0_{20,w}$ and $(\partial\rho/\partial c)^0_\mu$ in the Svedberg relation yields a molecular weight of $(610 \pm 25) \times 10^3$, which is comparable with a mean value of $(573 \pm 20) \times 10^3$ determined from light-scattering (Table 3). On the basis of these molecular weights the 19 S complex is composed of 20–23 27000-M_r proteins.

The radius of the hydrodynamically equivalent sphere is obtained by substitution of the measured diffusion coefficient in the Stokes–Einstein equation (Koppel, 1974):

$$R_h = \frac{kT}{6\pi\eta^0 D^0_{20,w}}$$

with k the Boltzman constant, η^0 the solvent viscosity and T the absolute temperature. $R_h =$

Table 3. *Molecular weight of the 19 S protein complex*
Experimental details are indicated in the text.

Technique		M
Light-scattering, with benzene as standard		
Polarized light	$\lambda = 436$ nm	538000
	$\lambda = 546$ nm	560000
Unpolarized light	$\lambda = 436$ nm	597000
	$\lambda = 546$ nm	596000
Svedberg relation		610000

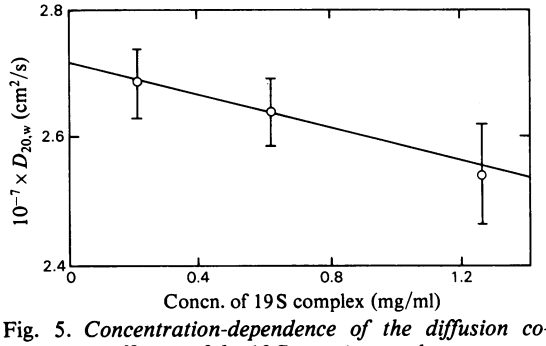

Fig. 5. *Concentration-dependence of the diffusion coefficient of the 19 S protein complex*
Diffusion coefficients were measured at 25°C from single clipped photon-count autocorrelation spectra. The 19 S protein samples in buffer Q were concentrated by ultrafiltration. Aggregates were removed by chromatography on Sepharose 4B and freed of dust by centrifugation at 15000 *g* for 2 h. Diffusion coefficients were corrected to standard conditions. The protein concentration is calculated from the absorbance at 280 nm.

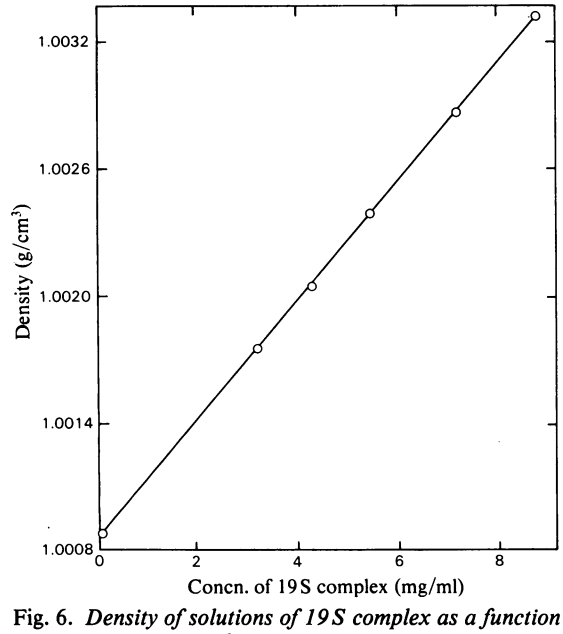

Fig. 6. *Density of solutions of 19 S complex as a function of concentration*
Purified 19 S protein complex was concentrated by ultrafiltration and extensively dialysed against buffer C. Samples were diluted with dialysis buffer and the density was measured at 25°C. The concentration of 19 S complex was calculated from the absorbance at 280 nm.

7.9 ± 0.2 nm (79 ± 2 Å) is in agreement with a diameter of 15.0 nm (150 Å) determined from electron-microscopic data (De Herdt *et al.*, 1980). From the volume of the hydrodynamic particle:

$$V_h = \frac{4\pi}{3} R_h^3 = (2.0 \pm 0.1) \times 10^3 \text{ nm}^3$$

$$[(2.0 \pm 0.1) \times 10^6 \text{ Å}^3]$$

and the volume of the dry particle:

$$V_d = \frac{v^0 M}{N} = (7.3 \pm 0.3) \times 10^2 \text{ nm}^3$$

$$[(7.3 \pm 0.3) \times 10^5 \text{ Å}^3]$$

the maximal weight of solvent per unit weight of the 19S complex can be calculated:

$$\delta = \frac{V_h - V_d}{v^0 (M/N)}$$

$$= 1.25 \pm 0.08 \text{ g of water/g of protein.}$$

Approximately the same hydration value was obtained from the formulae presented by Squire & Himmel (1979). Because of the spherical conformation of the 19S complex the maximal hydration is close to the real hydration. A hydration of 1.02 ± 0.02 g of water/g of protein is calculated from the radius determined from electron micrographs. These values are 1.5–2.0-fold higher than those for other proteins (Squire & Himmel, 1979).

Effect of 19S complex on mRNA translation

The 19S complex is one of the major RNA-binding protein complexes present in the cytoplasm of *Artemia salina* (De Herdt *et al.*, 1980). In other systems it has been shown that RNA-binding proteins are functional as protein-synthesis factors (Elizarov *et al.*, 1978; Ovchinnikov *et al.*, 1978; Vlasik *et al.*, 1978). Therefore the effect of the 19S complex was investigated on the translation of mRNA in a rabbit reticulocyte-lysate protein synthesizing system. The absence of the complex from the lysate was first checked by immunoprecipitation with anti-(19S protein complex) antibody. No effect was observed on the translation of poly(A)-lacking and poly(A)-containing mRNA isolated from *Artemia salina* (Figs. 7*a* and 7*b*). Tobacco-mosaic-virus RNA and globin mRNA were slightly inhibited by the 19S complex (Figs. 7*c* and 7*d*). An inhibition of only 35% was observed at a concentration of 19S complex of 0.6–0.8 mg/ml. This concentration is too high to assign a specific mRNA-inhibitory function to the 19S complex.

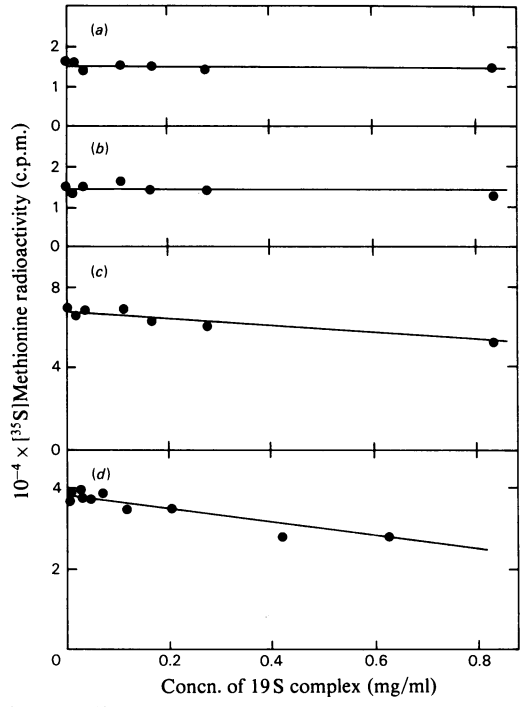

Fig. 7. *Effect of the 19S protein complex on mRNA translation in vitro in a rabbit reticulocyte lysate*

A constant amount of (*a*) 3 μg of *Artemia salina* poly(A)-lacking mRNA, (*b*) 0.8 μg of *Artemia salina* poly(A)-containing mRNA, (*c*) 1.2 μg of tobacco-mosaic-virus RNA and (*d*) 0.8 μg of rabbit globin mRNA was translated in the presence of increasing amounts of purified 19S complex. Acid-precipitable [^{35}S]methionine radioactivity was measured after incubation at 30°C for 50 min.

Discussion

The presence of a unique 19S complex has been observed in *Artemia salina* cryptobiotic embryos (De Herdt *et al.*, 1979*a*). This complex is located in the post-ribosomal supernatant and is the major component of this fraction. An amount of 3.6 mg/g of cysts is measured by immunoprecipitation with anti-(19S protein complex) antibody. The number of 19S proteins is 3 times that of ribosomes.

The quantity of this protein complex decreases drastically during the development of the cryptobiotic embryos to free-swimming nauplii and it is completely absent from 7-day-old larvae.

The 19S complex is a homoprotein complex composed of a 27000-M_r protein and has been partially characterized (De Herdt *et al.*, 1979*a*). From light-scattering experiments a molecular weight of 573000 is measured, comparable with a value of 610000 calculated from hydrodynamic parameters. On the basis of the molecular-weight

measurements, 20–23 27000-M_r proteins constitute the 19S homo-complex. Analysis of the proteins synthesized by the stored poly(A)-containing and poly(A)-lacking mRNA revealed that the 27000-M_r protein is not among the proteins synthesized *in vitro*. The decrease during development as well as the absence of synthesis by stored mRNA indicate the cryptobiosis-specific nature of this complex.

Cryptobiotic embryos of *Artemia salina* are characterized by a reversible dehydration that results in an arrest of development at the gastrula stage (Dutrieu, 1960; Morris, 1971). During desiccation two hydration thresholds have been measured. At a hydration of 0.65g of water/g of cysts, conventional metabolism decreases and only interfacial pathways are operative. Below 0.30g of water/g of cysts no metabolic activity is demonstrated (Clegg, 1978). Many macromolecules irreversibly lose their biological activity on removal of water, indicating the necessity of cellular components preventing complete dehydration of macromolecular structures for survival. Different low-molecular-weight components have been found in high concentration in cryptobiotic embryos, i.e. glycerol, trehalose and diguanosine tetraphosphate, each having a specific function in the post-gastrula development of the embryos (Clegg, 1974). Glycerol, present at a concentration of 2–6% of the dry weight, has a potential role in the stabilization of macromolecular structures during the desiccation process (Clegg, 1964, 1974). Clegg (1978) has compared the hydration of different components of *Artemia salina* before and after dehydration. Glycerol has the highest hydration of 1.86g of water/g of cysts, which decreases to only 0.074g of water/g of cysts after desiccation. By contrast, proteins, which are the major components (0.48g/g of cysts), have a hydration of 0.40 and 0.192g of water/g of cysts before and after desiccation respectively. The latter value is substantially higher than the one measured for glycerol. In cryptobiotic embryos the total amount of water retained by proteins is 51.3%, compared with only 19.2% retained by glycerol. More than 90% of these proteins are located in the outer shell and in yolk granules (Von Hentig, 1971; Warner *et al.*, 1972). The 19S protein complex represents only 0.8% of the total protein, but comprises 12–13% of the proteins in the post-ribosomal supernatant (Slobin, 1980). Furthermore, a very high hydration value of 1.02–1.25g of water/g of protein has been measured from hydrodynamic data. This value is at least twice that normally measured for proteins (Squire & Himmel, 1979). The high hydration value suggests a role in the water requirement for biological integrity of the cytoplasm during cryptobiosis.

The 19S complex has several other noteworthy biochemical properties: (i) a structural relationship with eEF-Ts factor has been demonstrated (De Herdt *et al.*, 1979*a*; Slobin, 1980); (ii) the 19S particle is one of the major RNA-binding proteins, but has no obvious function in the formation of messenger ribonucleoprotein (De Herdt *et al.*, 1979*a*, 1980; Slegers *et al.*, 1979); (iii) it has no function in mRNA translation, although inhibition of heterologous mRNA is observed at a concentration of 0.6–0.8mg/ml (Fig. 7).

The abundant presence of the 19S protein complex in cryptobiotic embryos and the absence of its synthesis during the further development indicate a role during the cryptobiotic process in early embryogenesis. We may postulate that the 19S complex is functional in the protection of cytoplasmic RNA-containing components against complete desiccation and/or maintaining the water content of the cytoplasm above a critical threshold.

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